

Claims

1. Expression cassette, characterized in that under the genetic control of an organ-specific or tissue-specific promotor and optionally one or more other regulatory elements 3'-downstream of the promotor, it encompasses the coding nucleotide sequence of at least one non-immunogenic receptor which is located on the cell surface.

2. Expression cassette as claimed in claim 1, wherein the coding nucleotide sequence codes for a receptor which has at least one of the following properties:

- a) it is not recognized in expressed form by the immune system of a mammal as "alien";
- b) it is not expressed under native conditions on the undifferentiated pluripotent precursor cells of the mammal;
- c) it is essentially not expressed by the organ-specific or tissue-specific cells of a mammal which are to be genetically modified using the expression cassette, under native conditions.

3. Expression cassette as claimed in claim 1 or 2, wherein the coding nucleotide sequence of the receptor is contained in a poly-cistronic gene which moreover comprises the coding sequence for at least one marker gene and/or a first therapeutic gene.

As a2 4. Expression cassette as claimed in one of the preceding claims, wherein the receptor has affinity for a ligand.

5. Expression cassette as claimed in claim 4, wherein the receptor is a surface antigen which has immunological affinity for an optionally immobilized immunoglobulin molecule.

6. Expression cassette as claimed in claim 5, wherein the surface antigen is a CD4 antigen or shortened fragment thereof, preferably its extracellular and transmembrane domain.

Inc 3 7. Expression cassette as claimed in one of the preceding claims, wherein it moreover comprises a reversibly integrated resistance gene.

8. Expression cassette as claimed in claim 7, wherein the resistance gene is flanked by LoxP sequences.

9. Expression cassette as claimed in claim 2, wherein the marker gene is the EGFP gene.

Inc 4 10. Expression cassette as claimed in one of the preceding claims, wherein it moreover comprises a second therapeutic gene.

11. Expression cassette as claimed in claim 2 or 10, wherein the first and the second therapeutic gene are chosen independently of one another from among genes for angiogenesis factors, such as especially the vascular endothelial growth factor (VEGF) gene, the basic fibroblast growth factor (bFGF) gene, the acidic fibroblast growth factor (aFGF) gene, the angiopoietin, activin and follicostatin gene, and immune suppression genes, such as especially the CTLA4-Ig fusion gene.

Inc 5 12. Expression cassette as claimed in one of the preceding claims, with coding sequences which code essentially for human or humaized gene products.

13. Expression cassette as claimed in one of the preceding claims, wherein the ventricle-specific myosin-light chain-2 (MLC-2v) promotor is used as the organ-specific or tissue specific ~~promotor~~

14. Expression cassette as claimed in claim 13, wherein it comprises in the 5'-3' direction at least one of the following partial sequence successions:

a) MLC-2v promotor, CD4 extracellular and transmembrane domains, IRES, angiogenesis factor;

b) CMV enhancer, MLC-2v promotor, CD4-extracellular and transmembrane domains, IRES, angiogenesis factor;

c) CMV enhancer, MLC-2v promotor, CD4 extracellular and transmembrane domains, IRES, angiogenesis factor, PGK promotor, CTLA4-Ig fusion protein; and

d) CMV enhancer, MLC-2v promotor, CD4-extracellular and transmembrane domains, IRES, angiogenesis factor, LoxP, PGK promotor, resistance gene, LoxP, PGK promotor, CTLA4-Ig fusion protein.

15. Expression cassette as claimed in one of claims 13 to 14, with coding sequences which code essentially for human or humanized gene products.

16. Vector comprising an expression cassette as claimed in one of claims 1 to 12.

17. Vector comprising an expression cassette as claimed in one of claims 13 to 15.

18. Process for isolation of in vitro differentiated organ-

a) an organ-specific or tissue-specific expression vector as claimed in claim 16 being introduced into pluripotent precursor cells, especially chosen from among embryonal stem cells, primordial cells and bone marrow stroma cells of a mammal;

c) optionally present resistance genes being removed from the selected cells;

d) in the cells obtained in this way, differentiation into a cell population comprising the desired organ-specific or tissue-specific somatic cells being induced and if necessary a single cell preparation being produced; and

e) the receptor-expressing differentiated somatic cells being affinity-purified using receptor-specific ligands.

a) the ventricle-specific expression vector as claimed in claim 17 being introduced into pluripotent precursor cells, especially chosen from among embryonal stem cells, primordial cells and bone marrow stroma cells of a mammal;

c) optionally present reversibly integrated resistance genes being removed from the selected cells;

d) in the cells obtained in this way, differentiation into a cell population comprising the cardiomyocytes being induced and if necessary a single cell preparation being produced; and

e) the receptor-expressing differentiated ventricular

cardiomyocytes being affinity-purified using receptor-specific ligands.

20. Process as claimed in claim 18 or 19, wherein LoxP-flanked resistance genes are used as reversibly integrated resistance genes and are transiently transfected to remove this expression vector which codes the cells with Cre recombinase.

21. Process as claimed in one of claims 18 to 20, wherein the embryonal stem cells are obtained from

- a) blastocysts or
- b) enucleated oocytes into which the nucleus of an differentiated adult somatic cell has been transferred.

22. Process as claimed in one of claims 18 to 21, wherein the receptor-specific ligands are coupled to paramagnetic microbeads and the ligand-marked cells are separated from the unmarked cells in a magnetic field.

23. Process as claimed in one of claims 18 to 22 for producing autologous human somatic cells, the pluripotent precursor cells being obtained from an autologous human donor.

24. Transgenic cardiomyocytes with an electrophysiologically ventricular property spectrum.


25. Transgenic cardiomyocytes as claimed in claim 24, with one, several or all of the following electrophysiological features:

- a) membrane potential in the range of roughly -68.6 ± 2.8 mV;
- b) membrane potential length in the range of roughly 118.3

± 15.2 mV;

- c) overshoot in the range of roughly 34 ± 3.9 mV;
- d) no response of the membrane potential and action potential length to $1 \mu\text{M}$ carbachol; and
- e) prolongation of the action potential by administering $0.1 \mu\text{M}$ isoprenaline.

26. Transgenic cardiomyocytes as claimed in claim 25, containing at least one vector as claimed in claim 17.

See 28  27. Transgenic cardiomyocytes which can be obtained using a process as claimed in one of claims 19 to 23.

28. Transgenic cardiomyocytes which can be obtained using a process as claimed in claim 18 or 20 to 23.

29. Use of transgenic cells as claimed in one of claims 24 to 28, for preferably autologous cell transplantation, or for gene therapy, as especially for cell-mediated gene transplantation.

30. Use of an expression cassette as claimed in one of claims 1 to 15 or of a vector as claimed in claim 16 or 17 for genetic alteration of pluripotent precursor cells of a mammal.

31. Use of an expression cassette as claimed in one of claims 1 to 15 or of a vector as claimed in claim 16 or 17 for producing in vitro differentiated somatic cells of a mammal.

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